Supplementary Materials

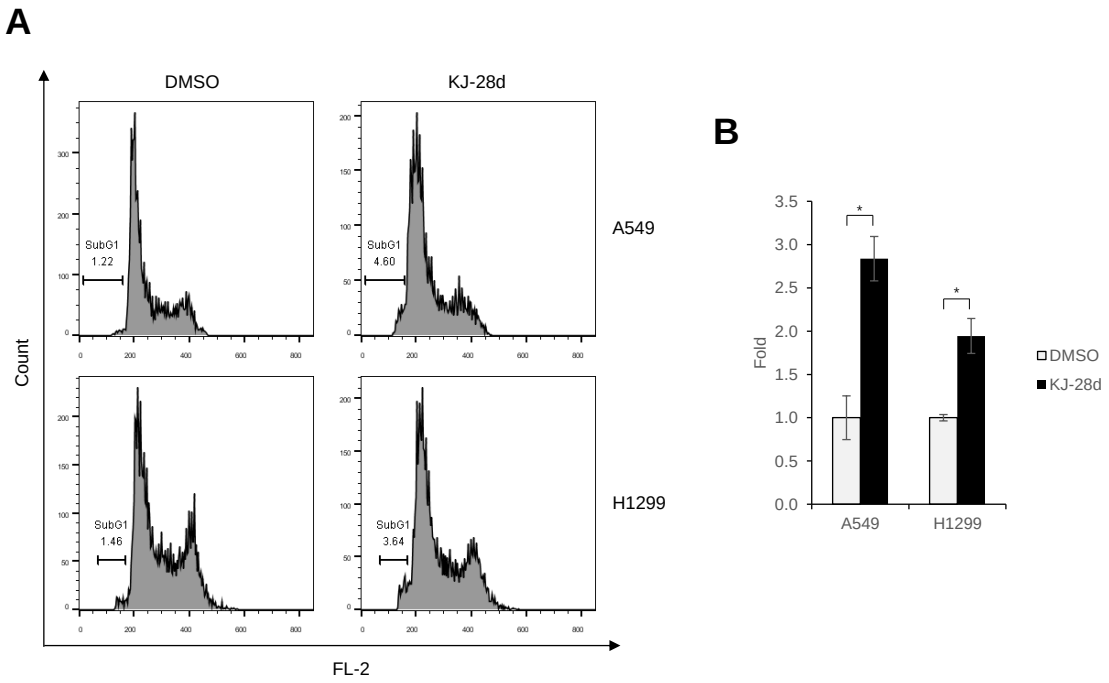

Figure S1. KJ-28d induces sub-G1 phase in A549 and H1299 cells. Human NSCLC A549 and H1299 cells were treated with 5 μM KJ-28d for 24 h and stained with PI. The cell cycle distribution was analyzed by flow cytometry (**A**). The bar graph shows the quantitative analysis of FACS data (**B**). **p* < 0.05 versus corresponding values.

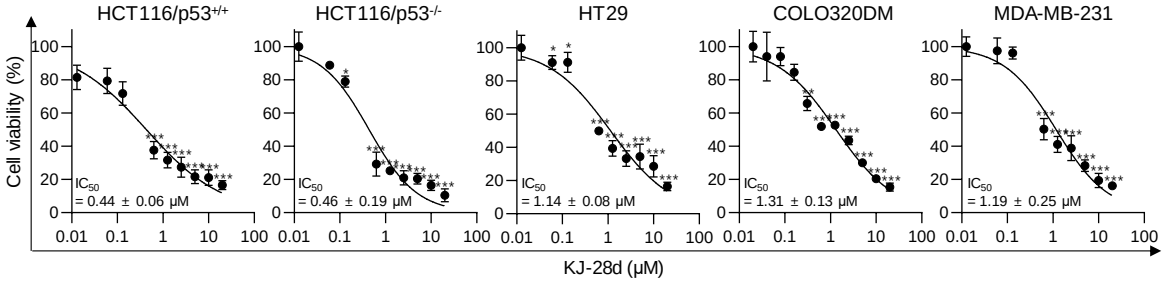

Figure 2. KJ-28d inhibits the proliferation of human cancer cells. Human colorectal cancer $p53^{+/+}$ HCT116, $p53^{-/-}$ HT29, and COLO320DM cells and human breast cancer MDA-MB-231 cells were treated with KJ-28d at indicated concentrations for 5 days and cell viabilities were determined using the MTT assay. Data are presented as means ± standard deviation (SD) from at least three independent experiments. **p* < 0.05, ***p* < 0.01, ****p* < 0.001 versus DMSO-treated control. .

Table 1. NSCLC cell lines used in this study.

Cell line	KRAS	EGFR	TP53	KEAP1	Remarks
A549	MT (missense) 34G>A, G12S	WT	WT	MT (missense) 997G>T, G333C	c-MYC: amplified
H1299	WT	WT	Null	WT	-
H1650	WT	MT (deletion) c2235-2249del, E746-A750del	c.673-2A>G	WT	TP53: deletion in intron
H460	MT (missense) 183A>T, Q61H	WT	WT	MT (missense) 706 G>C, D236H	c-MYC: amplified

Note: Mutation status of each cell line was determined from the Sanger Catalogue of Somatic Mutations in Cancer database (COSMIC, http://www.sanger.ac.uk/genetics/CGP/cosmic). Abbreviation: WT, wild-type; MT, mutation

Table S2. Activities (%) of KJ-28d (5 μM) against HDAC isoenzymes *in vitro.*

HDAC isoenzyme	Activity (%)
DMSO	100.0
HDAC1	82.4
HDAC2	100.0
HDAC3	100.0
HDAC4	98.4
HDAC5	92.5
HDAC6	78.3
HDAC7	100.0
HDAC8	83.2
HDAC9	100.0
HDAC10	100.0
HDAC11	78.3

Supplementary Materials and Methods

Sub-G1 analysis. Cells were treated with 5 μM KJ-28d. After 24 h treatment, cells were fixed in 70% cold ethanol overnight. For cell cycle analysis, fixed cells were treated with RNase for 20 min before addition of 5 μg/mL PI and analyzed by flow cytometry (CyFlow cube 6).

Cell culture. Human breast cancer cells (MDA-MB-231) and human colon cancer cells (HT29 and COLO320-DM) were obtained from ATCC (American Type Culture Collection). $p53^{+/+}$ HCT116 and $p53^{-/-}$ HCT116 cells were kindly provided by Dr. Bert Vogelstein (Johns Hopkins University, Baltimore, MD, USA). Cells were maintained in Roswell Park Memorial Institute (RPMI) 1640 medium (Welgene) supplemented with 10 % fetal bovine serum (FBS; Welgene) and 100 units/mL penicillin streptomycin solution (Gibco) at 37 °C in a humidified 5 % CO_2 atmosphere.

In vitro enzyme assay. Enzyme activities of KJ-28d against HDAC isoenzymes (1~11) were performed Reaction Biology Corp. (Malvern, PA, USA).